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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Tongtong Wang, et al.
Application No.	:	09/519,642
Filed	:	March 6, 2000
For	:	COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF LUNG CANCER
Examiner	:	Michael Borin
Art Unit	:	1631
Docket No.	:	210121.478C4

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF GARY FANGER, PH.D.

The undersigned, Gary Fanger, Ph.D., hereby declares:

1. I am a Scientist at Corixa Corporation, the assignee of the subject application. The following experiments were performed under my supervision.

2. In order to confirm L552S protein expression in various normal lung and lung cancer tissues, immunohistochemistry (IHC) analysis was performed using an affinity purified L552S polyclonal antibody. Specifically, tissue samples were fixed in a formalin solution for 12-24 hrs and embedded in paraffin before being sliced into 8 micron sections. Steam heat induced epitope retrieval (SHIER) in 0.1 M sodium citrate buffer (pH 6.0) was used for optimal staining conditions. Sections were incubated with 10% serum/PBS for 5 minutes. Primary antibody was added to each section for 25 minutes at indicated concentrations followed by a 25 minute incubation with an anti-rabbit biotinylated antibody. Endogenous peroxidase activity was blocked by three-1.5 minute incubations with hydrogen peroxidase. The avidin biotin complex/horse radish peroxidase (ABC/HRP) system was used along with DAB chromogen to visualize L552S expression. Slides were counterstained with hematoxylin to visualize cell nuclei. L552S polypeptide expression was detected in 10/12 lung adenocarcinoma samples, whereas no

expression was detected in 13 normal lung samples evaluated. This expression pattern establishes that L552S polypeptides are overexpressed in lung cancer tissue as compared to normal lung tissues and supports the use of the L552S polypeptides as a cancer diagnostic marker.

3. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Gary Fanger, Ph.D.

9/1/04
Date

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I hereby certify that on the date specified below, this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, DC 20231.

Date Sandi Duncan

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Tongtong Wang et al.
Application No.	:	09/589,184
Filed	:	June 5, 2000
For	:	COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF LUNG CANCER
Examiner	:	M. Borin
Art Unit	:	1631
Docket No.	:	210121.478C8
Date	:	

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D. C. 20231

DECLARATION OF ROBERT HENDERSON, Ph.D.

The undersigned, Dr. Robert Henderson, hereby declares:

1. I have been a Scientist and Group leader at Corixa Corporation, for the past four years and am now Director, Product Management—Cancer Vaccines for the assignee of the subject application. The following experiments were performed under my supervision, while I was a Group Leader.

2. Experiments were performed to confirm that L552S polypeptides are capable of detecting antibodies in lung cancer patients and to further identify, using routine techniques, illustrative immunogenic fragments of L552S polypeptides recognized by L552S-specific antibodies. More specifically, the presence of antibodies against L552S in effusion fluid obtained from lung cancer patients and in sera from normal donors was examined by ELISA using recombinant proteins and HRP-conjugated anti-human Ig. Briefly, L552S protein (100 ng) was coated onto a 96-well plate at pH

9.5. In parallel, BSA (bovine serum albumin) was also coated as a control protein. The signals (absorbance measured at 405 nm) against BSA were determined. Antibodies to L552S were detected in 7 of 20 samples of effusion fluid from lung cancer patients, with no L552S antibodies detected in the five samples of normal sera tested. Western blot analysis produced similar results. Antibodies against L552S were found to be present in 1 out of 4 samples of effusion fluid from lung cancer patients, with no L552S antibodies being detected in the three samples of normal sera tested.

Moreover, peptides corresponding to specific fragments of L552S were tested in order to confirm which portions of the polypeptide contain epitopes responsible for binding antibodies from the human lung cancer patient sera and/or effusion. Results from these experiments demonstrated that lung cancer patients produce L552S-specific antibodies recognizing the following immunogenic fragments of the L552S polypeptide encoded by SEQ ID NO:790:

(1) aa82-101: KVICKSCISQTPGINLDLGS
(2) aa107-126: IIPKEEHCKMPEAGEEQPQV
(3) aa21-40: ILSPLLRHGGHTQTQNHTAS

These results thus confirm the in vivo immunogenicity of L552S and further identify immunogenic fragments of L552S that are useful, for example, in testing patient sera for the presence of L552S-specific antibodies and/or for generating L552S-specific diagnostic antibodies.

3. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Robert Henderson, Ph.D.

9-17-2002
Date